Whey protein concentrate and skimmed milk powder as encapsulation agents for coffee silverskin extracts by spray drying

Letícia Ribeiro Barbosa, Júlia d'Almeida Francisquini, Ana Flávia Lawall Werneck Cerqueira, João Paulo Moreira, Luciana Poty Manso dos Santos, Elita Scio, Rodrigo Stephani, Ítalo Tuler Perrone, Humberto Moreira Húngaro and Mirian Pereira Rodarte

Supplementary File

Introduction

Coffee production for commercialisation as a raw bean generates approximately 45–50% waste, which is discarded and can cause various environmental problems (Gemechu, 2020). The high volume of residues has driven research into innovative alternative technologies to minimise the environmental impacts and process losses (Costa et al., 2014). During the roasting process, green coffee beans show an average weight loss of approximately 16%. In addition to water loss, this percentage includes the main by-product of this stage, coffee silverskin (Gemechu, 2020). This residue is an important source of bioactive compounds that can be used in food, cosmetics, and pharmaceutical products (Costa et al., 2018).

Coffee silverskin contains a lot of protein and a very low fat content and reducing carbohydrates are almost absent. The ash content is high, suggesting important mineral content. However, polysaccharides are the main components (Borrelli et al., 2004). Coffee silverskin also has a high content of dietary fibre (50-60%), which is divided into 15% soluble and 85% insoluble fibre (Borrelli et al., 2004). Previous characterisation studies have indicated that chlorogenic and caffeic acids are the main phenolic compounds present in coffee silverskin (Nzekoue et al., 2020). Its antioxidant capacity is also correlated with the production of melanoidins during roasting (Esquivel & Jiménez, 2012).

Spray drying is one of the most widely used microencapsulation methods in the food industry. It is a technique that transforms solutions or emulsions into products in the form of a powder and contains the material within a capsule to allow the isolation and protection of the compounds, thus guaranteeing their stability, promoting the maintenance of active principles during storage, and avoiding undesirable changes. Moreover, the advantages of this method include its simplicity, low cost, and flexibility, which allow continuous operation, high stability (low moisture content of powders), substantial volume reduction, ease of handling, and storage stability of the microparticles (Sun et al., 2019).

For microcapsules intended for food purposes, the encapsulating wall materials must be of food grade and easy to handle and must have low hygroscopicity and biodegradability (Rutz et al. 2013).

The encapsulation of phenolic compounds and antioxidants is an important strategy for preserving their properties for a longer time, as the encapsulating materials act as barriers to oxygen and water, thus improving their stability and enabling their use in the food industry (Lavelli, Harsha, & Spigno, 2016).

This study addressed the hypothesis that milk proteins, through microencapsulation, can guarantee protection against bioactive substances in coffee silverskin extracts. The aim of this study was to carry out technological, nutritional, and physicochemical characterisations of a coffee silverskin extract microencapsulated using SMP and WPC as wall materials.

Material and Methods

Preparation of extracts

To obtain the coffee silverskin extract, 1 g of ground and sieved sample was added to 50 mL of water, which was then heated to 50ºC on a hot plate with constant agitation (600 rpm) in a mechanical stirrer (model 715 W: Fisatom, Sao Paulo, Brazil). Subsequently, the extract was filtered under a vacuum pump through a Büchener funnel and Kitassato glassware, using 14 µm millex type filter paper. Extraction was performed in triplicate and extracts were stored at -25ºC before analysis (Costa et al., 2014). After the extraction process, part of the extract was frozen at -80ºC and freeze-dried (model LJJ04; JJ Científica, Sao Carlos, Brazil) for 24 hours, to be used as control. As a control, a portion of the extract was freeze-dried (Rutz et al., 2013).

Preparation of microcapsules for spray drying

Solutions of encapsulating agents (skimmed milk powder [SMP] and whey protein concentrate [WPC]) were prepared at a concentration of 10% (w/v). The resulting mixtures were homogenised using a mechanical stirrer (model 715 W; Fisatom) until complete solubilisation was achieved. Microencapsulation was performed using a spray dryer (Mini Spray Dryer B-290; Buchi) with inlet and outlet air temperatures of 162.5 ± 2.5°C and 82.5 ± 2.5°C, respectively. The obtained powders were packed in polyethylene bags containing a laminate layer and stored in the dark in a desiccator.

Water activity (a_w) and moisture content

The a_w was measured directly using an AquaLab 4TE instrument (Decagon Devices, Pullman, WA, USA) at 25 ± 1°C. Analysis was performed in triplicate. The moisture content of the samples was evaluated using an infrared moisture analyser (MOC63u; Shimadzu, Kyoto, Japan). For each sample, a mass of approximately 1.0 g was placed on an aluminium plate and heated at 105°C for a variable period of time, until reaching a constant mass. The percentage of mass loss was calculated as the moisture content of the sample. The assays were performed in triplicate.

Particle size analysis

The particle size distribution of the powders during the rehydration process was obtained using a Beckman Coulter LS 13320 laser diffraction analyser with an aqueous liquid module (Beckman Coulter, Brea, CA, USA). Sufficient quantities of samples to generate the turbidity required for the readings were added to the reservoir of the liquid analysis module containing water at room temperature. The data are presented as the percentage (%) of the volume occupied by the particles as a function of size. Beckman Coulter software (particle characterisation) version 5.03 was applied to analyse the data. The indicators Dv90 (volume in which 90% of the particles were found) and particles greater than 1.0 μm were used to assess the particle size distribution. Analyses were performed in duplicate.

Colorimetry

The colour of the powders was determined using a colorimeter (CR-400; Chroma Meter, Konica Minolta, Tokyo, Japan) with a direct reading of the reflectance of the coordinates L* (luminosity), a* (positive = red, negative = green), and b* (positive = yellow, negative = blue). Three measurements of each colorimetric parameter were performed with homogenisation of each sample during the storage period (Stangerlin et al., 2013).

The browning index (BI), used to evaluate the brown colour intensity of the samples, was calculated using Equation (1). The BI correlates the parameters L*, a*, and b* (Oliveira, Sousa-Gallagher, Mahajan, & Teixeira, 2012).

$$BI = \frac{[100(x - 0.31)]}{0.17}, \quad (1)$$

where $x = \frac{(a* + 1.750\ x\ L*)}{(5.645\ x\ L* + a* - 3.012\ x\ b*)}$

Total phenolics

The total phenol content was determined using the Folin–Ciocalteu spectrophotometric method, according to the methodology developed by Singleton and Rossi (1965), with modifications. Spectrophotometric absorbance was measured at 770 nm (UV–Vis 1601 Pc; Shimadzu). A gallic acid standard curve was constructed, and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of powder (mg GAE·100 g^{-1}).

Antioxidant activity

DPPH-based free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method described by Govindarajan et al. (2003) was used to determine the antioxidant potential of the samples, with some modifications. One hundred microlitres of the reference substance quercetin and the samples (1 mg/mL) were added to a 96-well plate at concentrations from 0.49 to 250 μg/mL (successive dilutions). Then, 150 μL of DPPH solution (20 μg/mL) in methanol was added to all wells containing the sample and reference substance. The reference compound (quercetin) and coffee silverskin extract were solubilised in methanol. Extract samples encapsulated in SMP and WPC were solubilised in a mixture of water and methanol (1:9). Two negative controls were used, which contained all reagents, except for the sample that was replaced with methanol or H_2O/methanol (1:9). The plate was incubated for 30 min in the dark and the absorbance was determined at 517 nm using a microplate spectrometer (UV–Vis 1601 Pc, Shimadzu). The entire experiment was performed in triplicate, and the results are expressed as the half-maximal inhibitory concentration (IC_{50}) and percentage of inhibition.

Nitric oxide radical scavenging activity

The method described by Green et al. (1982), which involves indirect dosage using the Griess method, was used with some modifications,. For the assay, the samples and reference substances were solubilised in phosphate buffer and tested at concentrations ranging from 250 to 7.8 μg/mL (successive dilutions). All reagents except the sample were used as negative controls. In a 96-well plate, 62.5 μL of sodium nitroprusside (NPS, 10 mM) solubilised in phosphate buffer (10 mM, pH 7.4) and 62.5 μL of the samples (1 mg/mL) and reference substance were added (gallic acid, 1 mg/mL). After the addition of NPS, the plate was incubated at room temperature for 60 min. Then, 125 μL of Griess reagent was added, and the absorbance was measured at 540 nm (UV–Vis 1601 Pc, Shimadzu) after 10 minutes and the percentage of inhibition was calculated using the Equation 2.

$$\%\ inhibition = 100\ -\ (\frac{A1\ x\ 100}{A0}), \qquad (2)$$

where A0 is the absorbance of the control, and A1 is the absorbance of the treated sample.

Particle morphology

The morphology and particle size of the powder samples were evaluated using scanning electron microscopy (TM3000; Hitachi Ltd., Tokyo, Japan). The unprepared samples were deposited on the support of the microscope and evaluated at magnifications of 100×, 250×, 500×, and 1,000×.

Statistical analysis

Statistical analyses were performed using GraphPad Prism® (version 8.0; GraphPad, San Diego, CA, USA). The results are expressed as the mean ± standard error of the mean, the different groups were compared using the analysis of variance method, followed by Tukey's test. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Table 1 presents the particle size distribution, based on the percentage of particles smaller than 1 μm, and the Dv90 value, which indicates 90% of the particles with values equal to or less than the result obtained. The coffee silverskin extract encapsulated with WPC had a significantly ($p \leq 0.05$) lower Dv90 value when compared to coffee silverskin extract encapsulated with SMP. A higher Dv90 value indicates a lower powder reconstitution efficiency (Paula et al., 2020). Paula et al. (2020) found average Dv90 values of 51.11 to 171.18 in three of the treatments used. Higher values when compared to the Dv90 value were found for coffee silverskin extract encapsulated with WPC, which indicates poorer reconstitution of the powder.

There was no significant difference ($p \leq 0.05$) in phenolic compounds between the different encapsulated coffee silverskin extracts. Nzekoue et al. (2020) extracted coffee silverskin using different solvents, which directly influenced the total phenolic content. Their results ranged from 40.4 to 73.4 mg GAE/g. Smaller values were found by Zengin et al. (2020), who performed the extractions in an ultrasonic bath. Values for phenolic composition from 20.49 to 35.68 mg GAE/g were found. There are several phenolic compound extraction methodologies, since the solubility of the extract may vary according to the solvent used and also according to other variables, such as time, temperature, and agitation (Martuscelli et al., 2021, Alara et al., 2021). Vimercati et al. (2022) reported an approximate value of 6 mg GAE/g when analysing the phenolic composition of a coffee silverskin extracts encapsulated with gum arabic, maltodextrin, or egg albumin.

In the DPPH assay, an increase in the antioxidant capacity of the coffee silverskin extract encapsulated in WPC was observed. That is, the IC_{50} result showed a higher antioxidant potential for the coffee silverskin extract encapsulated with WPC (IC_{50} 9.53 ± 2.92) than the freeze-dried coffee silverskin extract (IC50 66.15 ± 6.66) and the coffee silverskin extract encapsulated with SMP (IC50 184.35 ± 33.85), in addition to being statistically equivalent to antioxidant potential of quercetin, the reference substance in the reaction. This was a better result than that the result reported by Calva-Estrada et al. (2018), who found that the microcapsules of natural and synthetic vanilla extract encapsulated with WPC had IC_{50} values of 36.20 ± 3.50 and 29.50 ± 0.09, respectively.

For the nitric oxide radical scavenging activity, the freeze-dried coffee silverskin extract inhibited 59.40 ± 3.15% at a concentration of 250 µg/mL, while the coffee silverskin extract encapsulated with SMP and WPC inhibited 57.73 ± 3.05% and 44.24 ± 0.76%, respectively, at the same concentration. There was a significant difference ($p \leq 0.05$) between the encapsulated coffee silverskin extracts.

The antioxidant activity of phenolic-protein conjugates depends on the method used and the nature of the phenolics participating in the reaction (Liu et al., 2019). Thus, the increase in antioxidant activity when associating milk proteins with the phenolic compounds present in the coffee silverskin extract using the DPPH method can be explained by the synergistic interaction between the molecules.

Figure 1 shows the scanning electron microscopy analyses of the freeze-dried coffee silverskin extract, coffee silverskin extract encapsulated in SMP, and coffee silverskin extract encapsulated in WPC. The freeze-dried coffee silverskin extract, observed in A, presented a completely different morphology, when compared to the particles dried by the spray-dryer. This morphology is typical of the freeze-drying process (Mahdavee Khazaei et al., 2014). According to Kuck and Noreña (2016), the structural rigidity caused by the frozen surface and the lack of water in the liquid state results in a porous structure without shrinkage, which is the main characteristic of freeze-dried foods. In contrast, samples B and C contained higher amounts of fine particles and maintained very similar sizes, and most particles had a dehydrated appearance. Particles encapsulated by the spray dryer showed a spherical surface with a wrinkled outer surface and some concavities, which are typical characteristics of this encapsulation method (Ballesteros et al., 2017).

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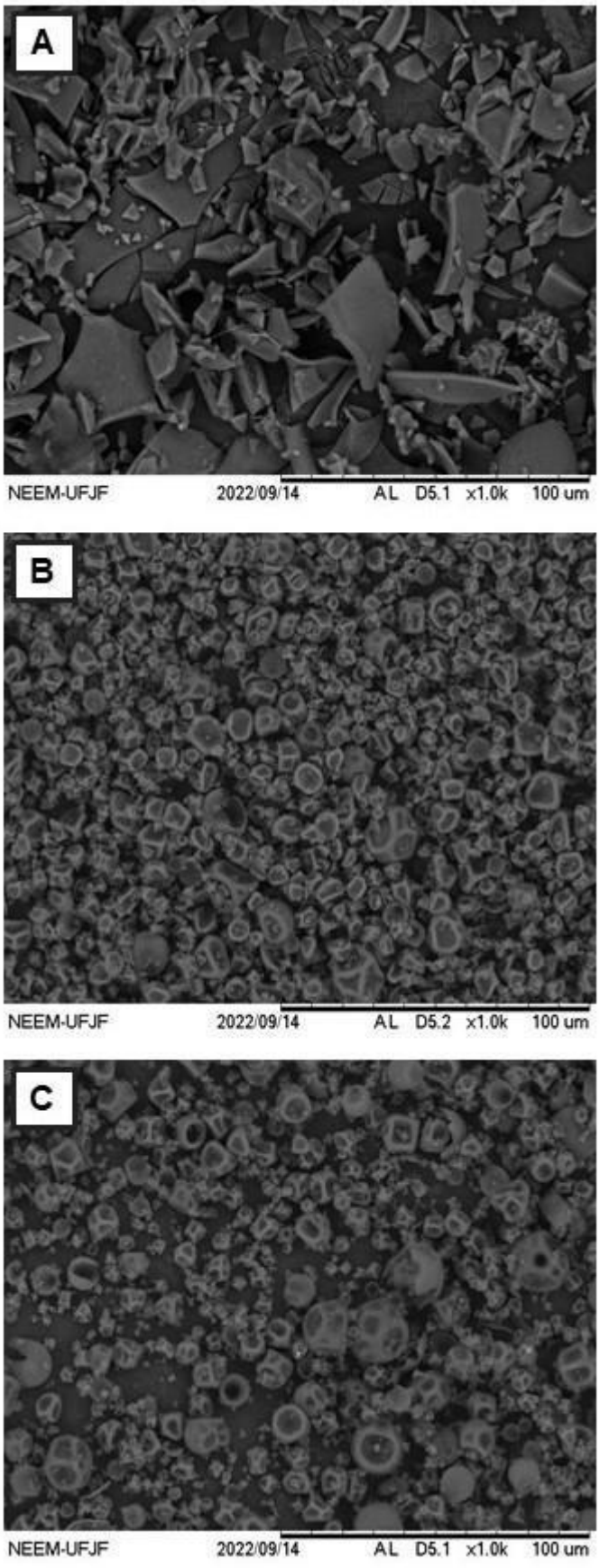
Figure legends:

Supplementary Figure S1:

Scanning electron microscopy analyses of the powders obtained from (A) freeze-dried coffee silverskin extract (B) coffee silverskin extract encapsulated with SMP (C) coffee silverskin extract encapsulated with WPC. Images at 1000x magnification.

Figure S1:


A
NEEM-UFJF 2022/09/14 AL D5.1 x1.0k 100 um
B
NEEM-UFJF 2022/09/14 AL D5.2 x1.0k 100 um
C
NEEM-UFJF 2022/09/14 AL D5.1 x1.0k 100 um